Alerts, Notices, and Case Reports

Pseudothrombocytopenia in a Child With the Acquired Immunodeficiency Syndrome

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THROMBOCYTOPENIA is a well-described finding in children with human immunodeficiency virus (HIV) infection.[1,2] Similarly, hematologic toxicity attributed to zidovudine therapy may include thrombocytopenia in addition to neutropenia and anemia.[3-5]

Herein we describe the case of an HIV-infected child (Centers for Disease Control classification P2) on zidovudine therapy with pseudothrombocytopenia due to ethylenediaminetetraacetate (EDTA)-induced chelation that was initially attributed to HIV infection, zidovudine toxicity, or both.

Report of a Case

The patient, a 6-year-old girl with neonatal transfusion-acquired HIV infection, was enrolled in the AIDS [acquired immunodeficiency syndrome] Clinical Trials Group Study No. 051 of the National Institute of Allergy and Infectious Diseases in September 1989. In the ten months preceding enrollment into the study, she had three documented episodes of thrombocytopenia (platelet counts ranging from 46 to 89×10^9 per liter [46,000 to 89,000 per μl]), two of which were associated with fever and an infectious process—pneumonia and *Streptococcus pneumoniae* bacteremia. Normal platelet counts were observed between these episodes, however. On the day a regimen of zidovudine, 180 mg per m^2 every six hours, and intravenous γ-globulin or albumin placebo (double-blind) was started, a peripheral blood smear revealed a platelet count of 59×10^9 per liter with evidence of platelet clumping on microscopic examination. Twelve days later, a platelet count of 296×10^9 per liter was recorded, and the low platelet count due to clumping was attributed to a suboptimal specimen collection. Over the next 12-month period, recurrent intermittent thrombocytopenia prompted a lowering of the zidovudine dosages, but the recurrent thrombocytopenia did not resolve. In addition, no clinical bleeding was ever noted in the patient. The possibility of pseudothrombocytopenia was considered when persistent platelet clumping became evident.

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To pursue the cause of the platelet clumping, blood was collected through a central line in the patient and simultaneously placed in a standard 5-ml EDTA-containing tube (Becton-Dickinson, Rutherford, New Jersey) and a 3-ml tube containing 0.5 ml of acid citrate dextrose (ACD) after informed consent was obtained from the parent. Complete blood counts were serially determined using a Coulter Counter Plus Jr automated cell counter over a two-hour period. Concomitant leukocyte histograms and smears were generated. Serum immune complexes were assayed by using a commercially available enzyme immunoassay (Diamedix, Miami, Florida). Platelet-associated immunoglobulin (Ig) G and IgM levels were determined with fluorescein-labeled anti-IgG and IgM antibodies and flow cytometry (Specialty Laboratories, Santa Monica, California).

Table 1 depicts the result of serial determinations of peripheral leukocyte counts and platelet counts for the EDTA- and ACD-anticoagulated specimens. Platelet counts of 152 and 161×10^9 per liter were initially noted in the EDTA- and ACD-anticoagulated specimens, respectively. After 105 minutes a rapid decrease in platelet counts to 42×10^9 per liter was noted in the EDTA-anticoagulated specimen but not in the ACD-anticoagulated specimen.

TABLE 1.—*Leukocyte and Platelet Counts in Specimens Anticoagulated With Ethylenediaminetetraacetate (EDTA) and Acid Citrate Dextrose (ACD)*

Time, min	Leukocyte Count, cells x 10^9/liter		Platelet Count, x 10^9/liter	
	EDTA	ACD	EDTA	ACD
0	3.8	2.9	152	161
15	4.4	3.0	98	157
35	5.4	3.2	60	153
60	5.7	2.9	49	154
75	5.8	3.0	45	161
105	5.7	3.1	42	149

The leukocyte counts rose from 3.8 to 5.8 cells $\times 10^9$ per liter over the 105-minute testing period in the EDTA-anticoagulated specimen, but remained in the range of 2.9 to 3.2 cells $\times 10^9$ per liter in the parallel ACD-anticoagulated specimen. Microscopic examination of the EDTA-anticoagulated peripheral blood smears revealed evidence of platelet clumping in all but the initial smear.

The associated neutrophil histograms revealed an abnormal leukocyte "shoulder" that increased with time but was not present in the ACD-anticoagulated specimen (Figure 1).

Serum immune complexes were not detected, although platelet-associated IgG and IgM were identified.

Discussion

Thrombocytopenia in HIV-infected children may result from immune-related phenomena,[1] splenic sequestration,[6] or antiretroviral medication.[3-5] Various therapeutic measures have included surgical (splenectomy) as well as medical (γ-globulin, steroid, dapsone, and antiretroviral) therapies.[6-8] It is therefore important to establish the cause of the thrombocytopenia to guide appropriate management and to avoid diagnostic and therapeutic misadventures. Pseudothrombo-

ABBREVIATIONS USED IN TEXT

ACD = acid citrate dextrose
AIDS = acquired immunodeficiency syndrome
EDTA = ethylenediaminetetraacetate
HIV = human immunodeficiency virus
Ig = immunoglobulin

cytopenia related to EDTA-anticoagulated blood has not to our knowledge been previously described in HIV-infected children and in this case resulted in unnecessary zidovudine dosage adjustment. The apparent rapid and dramatic decrease in platelet numbers noted in the EDTA-anticoagulated specimen but not in the parallel ACD-anticoagulated specimen confirmed the suspicion that the thrombocytopenia noted in our patient was artifactual. Pseudothrombocytopenia may be defined as a low platelet count due to laboratory artifact and may result from platelet satellitism,[9] giant platelet syndrome,[10] vasopressin infusion,[11] or an improper blood drawing technique.

Pseudothrombocytopenia has also been noted in patients with type IIB von Willebrand's disease[12] and in the serum of patients with antiplatelet antibodies.[13] Antibody-related pseudothrombocytopenia may result from cold agglutinins[14] as well as EDTA-dependent IgA, IgM, or IgG antiplatelet antibodies.[13,15]

Initially described by Shreiner and Bell,[16] the incidence of EDTA-induced platelet clumping has ranged from 0.09% to 1.9%.[17] Pegels and colleagues have demonstrated in vitro

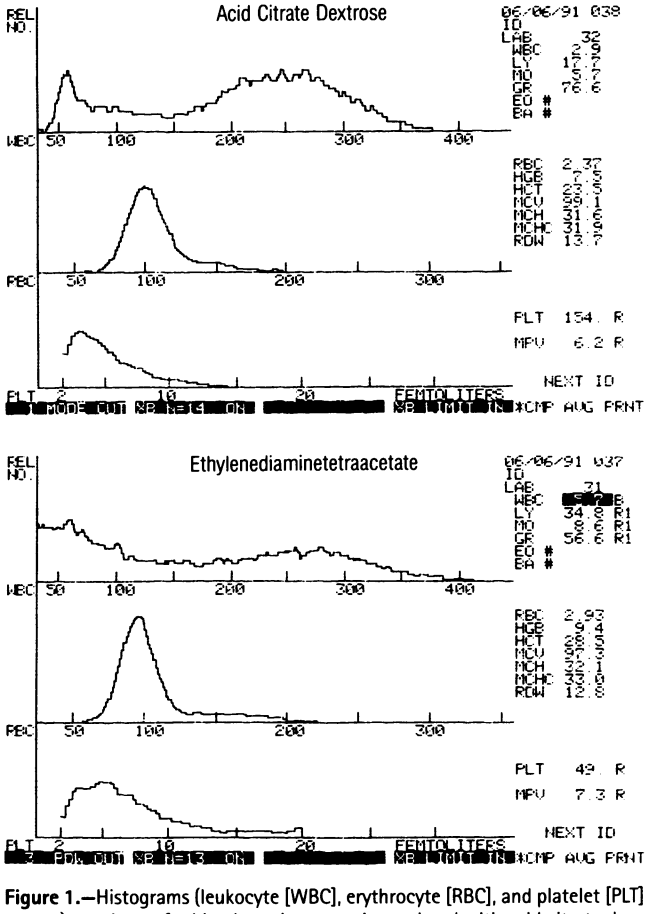

Figure 1.—Histograms (leukocyte [WBC], erythrocyte [RBC], and platelet [PLT] counts) are shown for blood specimens anticoagulated with acid citrate dextrose and ethylenediaminetetraacetate.

EDTA-dependent platelet agglutination due to IgG, IgA, and IgM antibodies in addition to EDTA-independent "cold" antibodies.[13] The EDTA-dependent platelet clumping occurred with EDTA concentrations as low as 0.3 mmol per liter and would react to platelets from normal donors but not in platelets lacking glycoproteins IIb or IIIa (or both), as in patients with Glanzmann's disease.

Older automated cell-counting devices—Coulter Model S and S-Plus—were unable to detect platelet clumping, whereas the Coulter Counter Plus Jr used in our laboratory showed a "high takeoff" or "shoulder" from the ordinate in the EDTA-anticoagulated specimen (which was not present in the parallel ACD-anticoagulated specimen). Conversely, a "leukocyte shoulder" may be present in the absence of platelet clumping.[18]

The initial intermittent thrombocytopenia present in our patient was not recognized as caused by platelet clumping, as large platelet clumps may have falsely simulated neutrophils, thereby failing to show a "shoulder" on the histogram. This possibility is supported by the apparent reciprocal increase in leukocyte counts, as measured by the Coulter Counter Plus Jr, with decreasing platelet counts in the EDTA-anticoagulated specimen. Of interest, pseudoleukocytosis associated with pseudothrombocytopenia has been described by others.[18,19]

Because EDTA-dependent pseudothrombocytopenia appears to be time dependent, as shown in our patient and by others,[15,19] the intermittent thrombocytopenia seen earlier in our patient's course may have been detected only when processing of the specimen was delayed.

In summary, the appearance of thrombocytopenia in an HIV-infected child should be evaluated for possible pseudothrombocytopenia due to EDTA-induced chelation. If an automated cell counter device is used, the presence of an early "shoulder" would suggest the possibility of platelet clumping. It should be remembered, however, that the leukocyte histogram "shoulder" may be absent despite platelet clumping. Consequently, microscopic examination of a peripheral blood smear from an ACD-anticoagulated specimen or from a finger-stick blood specimen would elucidate the presence or absence of platelet clumping and thereby avoid unnecessary medical and diagnostic misadventures.

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Reflections on the Anion Gap in Hyperglycemia

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THE ANION GAP is the difference between measured anions and measured cations; it reflects primarily the unmeasured anions, those not identified by the usual electrolyte determination.[1,2] An increased anion gap generally indicates the accumulation of organic anions. To calculate the gap, the following formula is often used:

$$\text{Anion gap} = [Na^+] - ([HCO_3^-] + [Cl^-]) = 8 \text{ to } 16 \text{ mEq/liter}$$

Electrolyte determination using some of the new ion-specific electrode methods may yield a slightly lower range for the anion gap but does not alter the principle.[3]

A case of a patient with hyperosmolar coma and associated electrolyte abnormalities prompted us to question how the anion gap should be calculated in such situations. Specifically, should its calculation be modified in any way to account for the dilutional effect of severe hyperglycemia on serum electrolyte levels? To our knowledge this issue has not been addressed previously in the medical literature.

To answer this question we reviewed the primary literature on the anion gap, the acid-base status in hyperglycemic coma, and the physiologic principles underlying the dilutional hyponatremia seen in severe hyperglycemia. In addition, we conducted an informal survey to determine how other house staff and faculty at our institution (Stanford [California] University Medical Center) approached this problem.

(Varon J, Jacobs MB, Mahoney CA: Reflections on the anion gap in hyperglycemia. West J Med 1992 Dec; 157:670-672)

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Report of a Case

The patient, a 56-year-old woman with a history of non–insulin-dependent diabetes mellitus, presented to the hospital after being found incoherent by a relative. On arrival at the emergency department, the patient was lethargic but responsive to painful stimuli. Her blood pressure was 140/86 mm of mercury, heart rate 110 beats per minute, and respirations 24 per minute. There were blood pressure and pulse orthostatic changes. Physical examination findings were unremarkable except for a depressed mental state. Admission laboratory studies revealed the following values: serum glucose 87.4 mmol per liter (1,574 mg per dl), sodium 111 mmol per liter (mEq per liter), potassium 5.7 mmol per liter, chloride 76 mmol per liter, bicarbonate 17 mmol per liter, blood urea nitrogen 26.7 mmol per liter (74.7 mg per dl), and creatinine 177 μmol per liter (2 mg per dl). An arterial blood gas determination with the patient breathing room air showed a pH of 7.35, a $Paco_2$ of 36 torr, and a Pao_2 of 82 torr.

Before the blood gas results were received, an attempt was made to analyze her acid-base status by calculating the anion gap. The admitting house staff was unsure as to whether and how this calculation should be done given the dilutional effect of profound hyperglycemia on the serum electrolytes.

Subsequently this case was presented to 33 house officers (internal medicine residents in postgraduate years 1 to 3) and 18 general internal medicine faculty. The written report was given, and they were asked to calculate the anion gap, to give the method for their calculations, and to analyze the acid-base status.

Results

Nearly a third of the faculty (5 [28%]) and a third of the house staff (11 [33%]) significantly exaggerated the magnitude of the anion gap by correcting the sodium concentration for the degree of hyperglycemia but neglecting to correct the other electrolytes. None of the house staff and only one faculty member corrected the anion gap for the dilution of all the electrolytes. Moreover, respondents uniformly misinterpreted an increased gap as synonymous with acidosis. Only 18 house staff (54%) and 6 faculty (33%) requested a blood gas analysis to confirm this impression.

Discussion

The central issue raised by this case is whether the calculation of the anion gap should be modified to correct for the dilutional effect of severe hyperglycemia on all the serum electrolytes, as is commonly done for the serum sodium level. To our surprise, we found that medicine house staff and faculty internists at our institution frequently exaggerate the anion gap in hyperglycemia by using a "corrected" value for the serum sodium but not for other electrolytes. Using two distinct lines of reasoning, we concluded that the anion gap should be calculated from the electrolytes as measured. The basis for this conclusion will be discussed further. In addition, we detected a common misconception that an increased anion gap is indicative of acidosis. The limitations of the anion gap in leading to this conclusion warrant review.

It is commonly understood that severe hyperglycemia is associated with a dilution of the serum sodium, which resolves as the glucose level is lowered. This phenomenon was first described by Seldin and Tarail in 1949.[4] Serum electrolyte levels are diluted by the movement of water out of cells